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An isotopic technique to mark mid-sized vertebrates non-invasively

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Abstract

Although dispersal is an important attribute of animal population ecology, knowledge of dispersal rates or distances for many wide-ranging species is lacking. Current methods require capturing and restraining animals, which can be cost-prohibitive, fail to collect sufficient samples or change animal ranging behaviors. Herein, we describe a novel, cost-effective and non-invasive method, using bait enriched with stable isotopes to mark the hair of American martens *Martes americana*. Captive martens that consumed isotopically labeled glycine exhibited significant and progressive enrichment in the isotopic signature of ^{13}C, ^{15}N and ^{2}H in both whole blood and hair. A distinct mark in hair, >2 standard deviations above natural abundance, occurred within 14 days of the second dose. The rate of isotopic labeling of hair was higher in spring, possibly because labeled amino acids became diluted among the many hairs growing during the autumn. Because hair and feathers can be collected non-invasively from large geographic areas without capturing animals, this labeling method can be used to mark and study the movement and dispersal rates of animals across landscapes efficiently.

Introduction

Dispersal is a fundamental aspect of animal life history and ecology; knowledge of it is necessary to develop effective conservation strategies (Macdonald & Johnson, 2001). However, quantifying dispersal rates and distances for wide-ranging species is challenging. Traditionally, methods such as mark–recapture, radio-telemetry and, more recently, DNA genotyping have been used to assess movements over long distances (Nathan *et al.*, 2003). Although generally accurate, these approaches require large investments of time, money and personnel, limiting the number of study animals and duration that each can be monitored. Such constraints reduce the likelihood of detecting rare, but important dispersal events (Nathan *et al.*, 2003). These methods also require capture, restraint and often anesthetization of animals, which can be stressful (Harper & Austad, 2000) and alter subsequent behaviors (e.g. Clinchy, Krebs & Jarman, 2001). Therefore, for wide-ranging vertebrates, particularly endangered ones, reliable estimates of dispersal are lacking, impeding our ability to parameterize population-based models for conservation (Macdonald & Johnson, 2001).

Naturally occurring stable isotopes have been used successfully to document long-distance movements of volant animals (Rubenstein & Hobson, 2004). For example, Cryan *et al.* (2004) used stable isotopes in hair to show that hoary bats *Lasiurus cinereus* can disperse ≥ 2000 km. Similarly, using isotopic signatures of feathers, Hobson & Wassenaar (1997) determined the breeding origin of wintering Neotropical migrants. These studies have largely relied on natural abundances of deuterium (^{2}H) across latitudinal gradients, which does not exhibit sufficient variation to determine the dispersal of animals moving short or intermediate distances (<100 km; Wunder & Norris, 2008). Previous researchers have, however, used organic and inorganic compounds, artificially enriched with stable isotopes, to track the fates of substances in various ecosystem (Hall & Tank, 2003) and physiological (Boutton, 1991; Hirons, Schell & St. Aubin, 2001) processes. Animal ecologists recently have applied these enriched isotopes as organismal markers: by dripping enriched nitrogen (^{15}N) into streams and ponds, Caudill (2003) and Macneale, Peckarsky & Likens (2004) marked and subsequently recaptured developing invertebrate larvae to estimate dispersal distances; similarly, Wanner *et al.* (2006) used ^{44}Ca to mark and track the dispersal and foraging activity of the parasitoid wasp *Cotesia glomerata*.

Vertebrate ecologists are increasingly using tissues collected non-invasively and DNA-based analyses to estimate dispersal and gene flow (DeYoung & Honeycutt, 2005). However, this approach can be limited by degradation of samples and related genotyping errors (Mills, 2007), which can increase bias of population estimates (Lukacs & Burnham, 2005). Such analytical errors could be avoided with the

use of an independent organismal marker. We reasoned that isotopically enriched compounds could also be used to mark free-ranging vertebrates. With artificially enriched baits and non-invasive sampling methods, researchers could mark animals and subsequently collect hair, feather or other tissues to track the movements of many individuals. For mammals and birds, in which most amino acids incorporated into hair and feathers are derived from recently consumed food (Ayliffe *et al.*, 2004), isotopic enrichment of ingested amino acids could rapidly mark keratinous tissues. No previous research has tested whether this approach can produce an unambiguous mark in tissues that can later be collected non-invasively.

As part of a project assessing the effects of fragmentation on the forest-associated mesocarnivore, the American marten *Martes americana*, we required a cost-effective and non-invasive method to investigate movement of individuals between habitat patches. By providing bait enriched with different isotopic labels to martens inhabiting isolated forest fragments and subsequently collecting hair samples non-invasively from a large area over multiple sampling periods, we intended to estimate dispersal rates and distances. Before using isotopically labeled baits in the field, we conducted feeding trials on captive martens to ascertain that consumption of an isotopically enriched diet would produce a reliable mark in the hair. Our specific objectives were to: (1) measure the dose-specific assimilation of isotopically labeled amino acids into marten blood and hair; (2) determine minimum dosages needed to label wild martens; (3) identify the season in which isotopically enriched bait would provide the most reliable mark.

Materials and methods

During the last week of March 2006, we captured five martens along snowmobile routes and logging roads in the Snowy Range Mountains of south-eastern Wyoming (41°4′N 106°9′W). We anesthetized captured martens with ketamine hydrochloride (15.0 mg kg^{-1} body weight) and xylazine hydrochloride (1.6 mg kg^{-1} body weight; Ben-David, Schell & Flynn, 1997) and transported them to individual outdoor pens at Red Buttes Environmental Biology Laboratory at the University of Wyoming. Here, captive martens were exposed to ambient changes in photoperiod and temperature, minimizing interference with their moult cycle and fur growth. They were fed *ad libitum* (Exclusive^{TM} cat food, PMI Nutrition®, Henderson, CO, USA) and had continuous access to water. Pens were furnished with PVC tubes, nest boxes, branches and small trees as environmental enrichment. Martens were acclimated to captivity and the cat food diet for 5 weeks. In the second week of May (coinciding with the moult; Soutiere & Steventon, 1981), we administered a unique combination of enriched (98–99 at.%) isotopes (^{13}C, ^{15}N and ^{2}H; ISOTEC^{TM}, Miamisburg, OH, USA) to the food of each animal ($n = 2$ for ^{13}C and ^{2}H, and $n = 3$ for ^{15}N); animals that did not receive an enriched diet for ^{13}C, ^{15}N or ^{2}H were used as controls for that isotope ($n = 3$ for ^{13}C and ^{2}H, and $n = 2$ for ^{15}N). For example, an animal that received a diet enriched in ^{2}H served as a control for ^{13}C and ^{15}N. Isotopic labels were provided in the form of glycine (Boutton, 1991), an amino acid that is incorporated into both blood and hair (Hirons *et al.*, 2001). We estimated the isotopic dose required to produce an isotopic label in the hair and blood of martens, assuming no elemental routing to different tissue types (i.e. complete mixing; Table 1). Specifically, we calculated the mass of an isotope ($^{\varepsilon N}A_g$) needed to elicit a significant increase in the signature of tissue with the equation:

$$^{\varepsilon N}A_g = [^{\varepsilon N}A \times {}^{N}A \times A_g] - [A_g \times {}^{N}A] \quad (1)$$

where $^{\varepsilon N}A$ is the isotopic enrichment desired (the proportion of labeled isotope above natural abundance). For example, our desired enrichment was 1.05 times natural abundance for ^{15}N, 1.04 times for ^{13}C and, because roughly 20% of hydrogen within hair exchanges with the atmosphere (Cryan *et al.*, 2004), 1.50 times for ^{2}H. ^{N}A is the natural abundance of the heavy isotope, and A_g is the mass of the element in the species (Table 1). To determine the mass of enriched glycine to feed martens, we multiplied the grams of enriched element to achieve the necessary mark by the molecular mass (M_r) and divided by the atomic mass (m_a) of that isotope:

Table 1 Parameters used to calculate the mass of glycine artificially enriched with ^{15}N, ^{13}C or ^{2}H to mark the hair of American martens *Martes americana*

Parameters	Nitrogen	Carbon	Hydrogen
Proportion of element in tissue	0.14	0.45	0.07
Grams of element in tissue (A_g)[a]	42	135	21
Proportion of heavier isotope in tissue (^{N}A)	3.70×10^{-3}	1.11×10^{-2}	1.56×10^{-4}
Grams of heavier isotope in tissue ($^{N}A_g$)[a]	0.156	1.499	0.003
Desired enrichment ($^{\varepsilon N}A$)	1.05	1.04	1.50
Grams of isotope needed ($^{\varepsilon N}A_g$)	7.78×10^{-3}	5.99×10^{-2}	1.64×10^{-3}
Atomic mass of isotope (m_a)	15	13	2
Molecular mass of enriched glycine (M_r)	76	76	80

Marten body mass was estimated as 1000 g, or 300 g dry mass (Buskirk & Harlow, 1989). Estimates of elemental and isotopic abundances are found in Rutherford & Hawk (1907) and Karasov & Martínez del Rio (2007).

[a]Values that are species-specific for American marten.

$$(M_r \times {}^{\varepsilon N}A_g)/m_a. \quad (2)$$

Finally, to calculate the dose of glycine that should be delivered to the animal, we multiplied this value by the fraction of animal dry mass (300 g) over its wet mass (1000 g).

We anesthetized each marten, plucked 10–20 newly growing hairs from the neck and rump, and collected 0.5 mL of blood from the jugular vein five times during spring (10 May–5 July) and six times during autumn (10 August–19 October). After the martens recovered from anesthesia they were fed the labeled food. Therefore, each marten received a dose of labeled glycine every 2 weeks, except between 5 July and 10 August. In autumn, to monitor hair growth, we also measured the length of the longest hair (cm) on the back, hind quarters, and middle of the tail on each anesthetized marten. During the final sample collection (19 October), we plucked 40–75 dorsal guard hairs from the tip of the tail of each marten to assess the temporal pattern of isotopic incorporation into hair. We elected to pluck these hairs, rather than those we measured on the middle of the tail, as they were longer (7.3 ± 0.22 cm long; mean ± 1 SD). Each guard hair was cut into six equal-length sections for isotopic analysis. All samples were stored frozen until prepared for mass spectrometry.

Samples of whole blood were thawed at room temperature, dried for 72 h at 60 °C and homogenized in a ball mill (Mixer Mill MM200, Retsch Inc., Newtown, PA, USA; MacAvoy, Macko & Arneson, 2005). Hair samples were rinsed three times with 2:1 chloroform:ethanol solution to remove surface oils (Cryan *et al.*, 2004), dried for 72 h at 60 °C and homogenized with surgical scissors. Samples were weighed, placed in tin (^{13}C and ^{15}N) or silver (^{2}H) capsules and submitted to the Stable Isotope Facility at the University of Wyoming. Analysis of ^{13}C and ^{15}N levels was conducted with a Costech 4010 elemental analyzer (Costech Analytical Technologies, Valencia, CA, USA) and ^{2}H with a Finnigan TC/EA (High Temperature Conversion Elemental Analyzer) attached to a Thermo Finnigan Delta^{PLUS} XP Continuous Flow Isotope Ratio Mass Spectrometer (Thermo Fisher Scientific, Inc., Waltham, MA, USA). Results are provided as per mil (parts per thousand [‰]) ratios relative to the international standards of Peedee Belemnite (PDB; $\delta^{13}C$), atmospheric nitrogen (AIR; $\delta^{15}N$) and Standard Mean Ocean Water (SMOW; $\delta^{2}H$) with calibrated internal laboratory standards. Because all samples were collected, processed and analyzed in the same geographic location we did not correct the raw ^{2}H data for atmospheric exchange (Wassenaar & Hobson, 2000). We considered hair and blood samples to be significantly enriched when values were elevated ≥ 2 SD above those of control animals.

To account for the lack of independence and to improve the precision of parameter estimates, we analyzed changes in the isotopic signature of blood and plucked body hair with random coefficient models (RCM). We treated time, season (spring or autumn) and treatment status (receiving isotopically labeled or unlabeled diet) as fixed effects, and individual identity as a random effect (Littell *et al.*, 1996). To model the growth of hair, we fitted a Gompertz curve to the proportional growth of tail hair through time (Rose *et al.*, 1984; Zullinger *et al.*, 1984). Because the onset of hair replacement is delayed on the tail relative to other parts of the body (Bassett & Llewellyn, 1949), we excluded the two measurements before 7 September from the curve fitting because they reflected the length of hair grown in the previous year (Supporting information Fig. S1). We used estimates of hair growth rates derived from the Gompertz curve to provide a temporal scale to the segments of cross-sectioned guard hairs.

Results

During both periods (spring and autumn) whole blood of martens fed isotopically enriched diets exhibited progressive isotopic enrichment, whereas the blood of control animals did not (Fig. 1; RCM: $^{13}C - F_{1,41} = 20.6$, $P < 0.001$, $^{15}N - F_{1,41} = 12.3$, $P = 0.001$ and $^{2}H - F_{1,41} = 4.44$, $P = 0.041$). For control animals, the only slope coefficient differing from zero was for ^{2}H in autumn (Table 2), but the slope was negative. For treatment animals, rates of isotopic incorporation were >1.5 times higher during spring than during autumn (Table 2). Following a 4-week lapse in isotopic dosing and sample collection (between 5 July and 10 August), enrichment of blood with ^{2}H returned to pre-dose levels; ^{15}N and ^{13}C remained only slightly elevated (Fig. 1).

Isotopic enrichment in plucked hair was similar to whole blood; martens fed enriched glycine exhibited elevated isotopic signatures compared with control animals, which demonstrated variable and non-substantive isotopic changes (Fig. 1; Table 2; RCM: $^{13}C - F_{1,41} = 11.0$, $P = 0.002$, $^{15}N - F_{1,41} = 8.1$, $P = 0.007$ and $^{2}H - F_{1,41} = 40.5$, $P < 0.001$). In contrast to blood, plucked hair exhibited delayed incorporation of isotopes (Fig. 1). Hair samples were not significantly enriched relative to controls following the first dose; an enrichment of 2 SD above control values was observed only after the second dose (58% for ^{13}C, 75% for ^{15}N and 82% for ^{2}H). The isotopic signature of hair did not, however, change substantively following the third dose (Fig. 1). Body hair demonstrated the greatest rate of enrichment during the spring; slope coefficients were higher by 4.6-fold for ^{13}C, 2.5-fold for ^{15}N and 3.9-fold for ^{2}H (Table 2). In contrast to blood, hair remained significantly enriched in both ^{13}C and ^{15}N during autumn from isotopic dosing that occurred the previous spring. The enrichment of labeled hair with deuterium declined by *c.* 20‰ between spring and autumn (Fig. 1), presumably because some keratin-bound hydrogen exchanged with the atmosphere.

Based on a Gompertz function of hair-growth ($r^2 = 0.606$, $F_{2,18} = 12.3$; $P < 0.001$; Fig. 2), the oldest segment at the tip of the hair shaft, plucked from the tail, was grown during the period 29 June–27 July (28 days), which is within the range of initiation dates reported for closely related taxa (Rose *et al.*, 1984; Maurel, Coutant & Boissin, 1987). The remaining segments were grown during 27 July–9 August (13 days), 9–21 August (12 days), 21 August–3 September (13 days), 3–21 September (18 days) and 21 September to

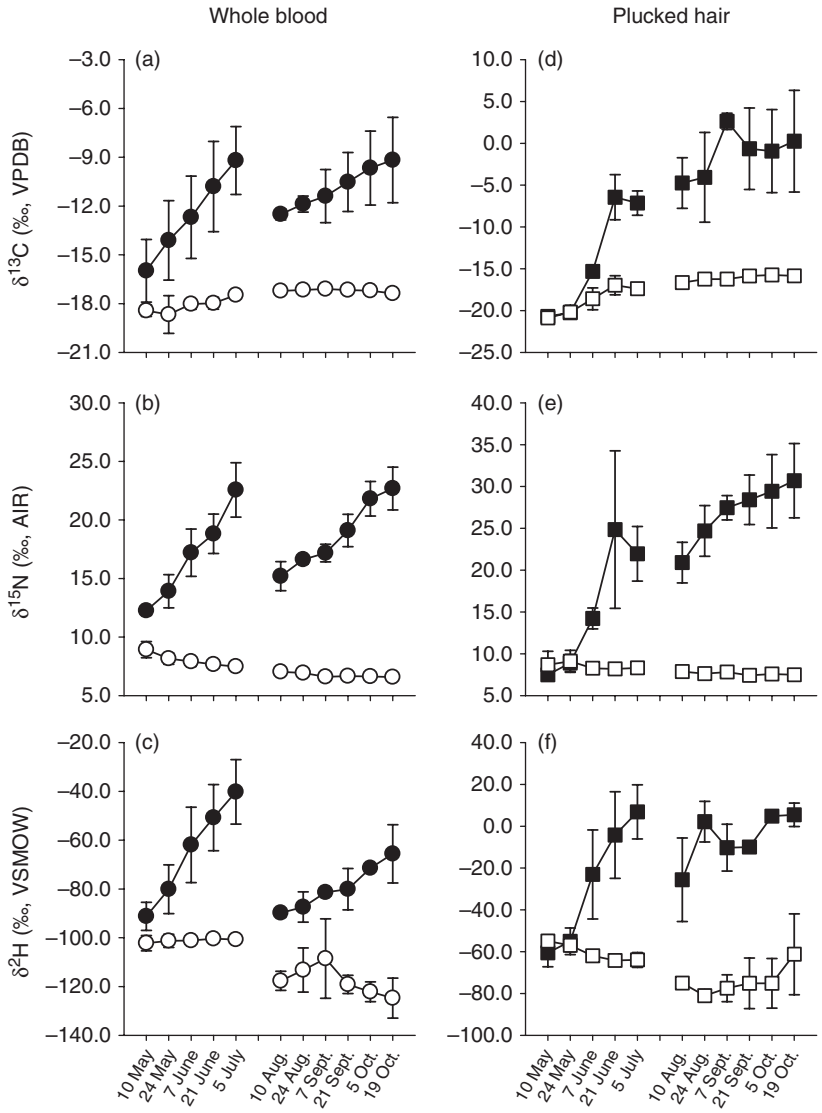

Figure 1 Mean isotopic values (±1 SD) of whole blood (circles; a–c) and plucked hair samples (squares; d–f) from captive martens, Laramie, Wyoming, 2006. Treatment animals (solid) were fed glycine isotopically labeled with ^{13}C, deuterium (^{2}H) or ^{15}N. Control animals (hollow) received an isotopically normal diet.

Table 2 Parameter estimates (slope and *y*-intercept; ±1 SE) from a random coefficient model for isotopic enrichment of blood and hair of captive American martens *Martes Americana* in spring (May–June) and autumn (August–October), 2007

	Whole blood				Hair			
	Spring		Autumn		Spring		Autumn	
Isotope	Slope	Intercept	Slope	Intercept	Slope	Intercept	Slope	Intercept
^{13}C – labeled	1.7 ± 0.2	−17.6 ± 0.5	0.7 ± 0.1	−13.3 ± 0.5	4.1 ± 0.5	−26.2 ± 1.5	0.9 ± 0.4	−4.4 ± 1.3
^{13}C – control	0.3 ± 0.1	−18.9 ± 0.4	−0.0 ± 0.1	−17.1 ± 0.4	1.0 ± 0.4	−21.9 ± 1.2	0.2 ± 0.3	−16.7 ± 1.1
^{15}N – labeled	2.6 ± 0.2	9.3 ± 0.7	1.6 ± 0.2	13.3 ± 0.6	4.5 ± 0.7	2.0 ± 1.7	1.8 ± 0.6	20.5 ± 1.6
^{15}N – control	−0.3 ± 0.2	9.1 ± 0.8	−0.1 ± 0.2	7.1 ± 0.8	−0.2 ± 0.8	9.0 ± 2.1	−0.1 ± 0.8	7.8 ± 1.9
^{2}H – labeled	13.1 ± 1.7	−104.3 ± 8.0	4.9 ± 1.3	−96.3 ± 7.6	18.5 ± 2.2	−82.9 ± 6.6	4.7 ± 1.8	−21.9 ± 6.0
^{2}H – control	0.4 ± 1.4	−102.3 ± 6.5	−2.1 ± 1.1	−110.3 ± 6.2	−2.5 ± 1.8	−52.9 ± 5.4	2.6 ± 1.5	−83.1 ± 4.9

Labeled martens were fed a dose of the amino acid glycine enriched with ^{13}C, ^{15}N or ^{2}H; control animals did not receive an enriched diet for that isotope.

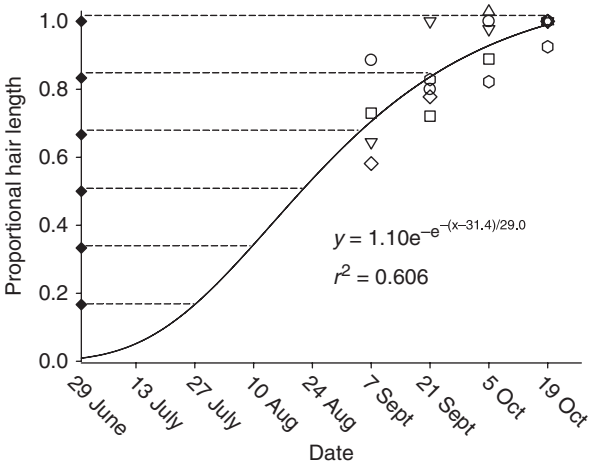

Figure 2 Gompertz function describing the growth rate of hair (following Rose *et al.*, 1984; Maurel *et al.*, 1987), based on measurements of guard hairs from the tails of five captive martens. This function was used to provide a chronology for growth of tail hair that was cross-sectioned and analyzed for isotopic signature (Fig. 3). Diamonds along the *y*-axis delineate the six segments of cross-sectioned tail hairs and the dotted lines provide location on the Gompertz function to estimate date of segment growth. Open symbols denote different individual animals. One observation (7 September, 4.7 cm) was excluded from modeling because it was an outlier.

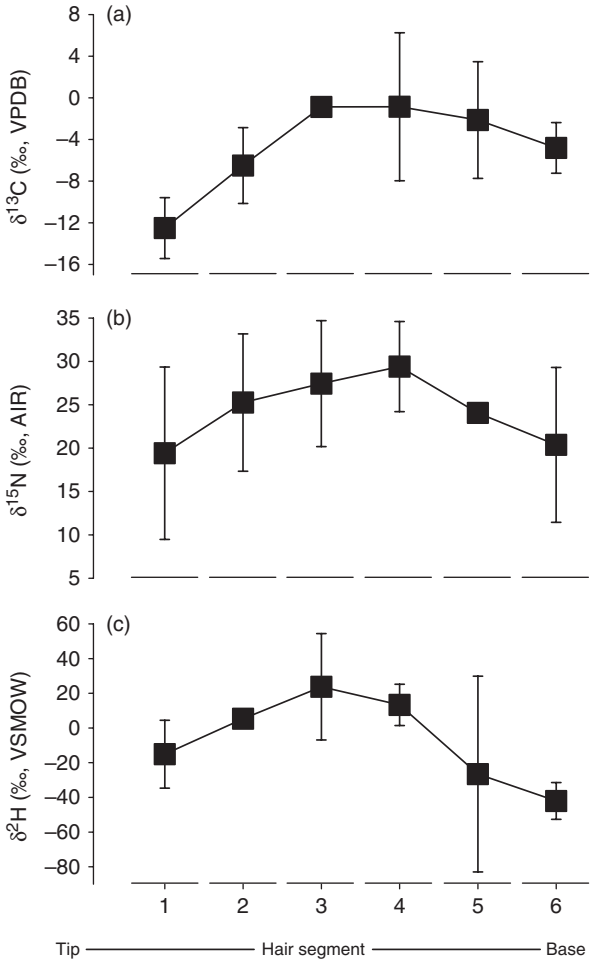

Figure 3 Mean isotopic values (± 1 SD) of dorsal guard hairs plucked from captive martens and cross-sectioned into six equal lengths, Laramie, Wyoming, 2006. Values shown are for martens fed glycine isotopically labeled with ^{13}C, ^{15}N or deuterium (^{2}H). Using a Gompertz function of tail growth (Fig. 2) we calculated the dates that each segment represented (1 = 13 July–2 August; 2 = 2–13 August; 3 = 13–24 August; 4 = 24 August–4 September; 5 = 4–20 September; and 6 = 20 September to the end of hair growth in October.

the time of collection of hair in October (29 days). Enrichment of cross-sectioned guard hairs from marten tails was highest in the middle segments of the hair shaft for all isotopes. The greatest enrichment was detected in the third segment for ^{13}C and in the fourth segment for ^{15}N and ^{2}H (Fig. 3). Thus, the highest enrichment in tail hair was obtained during August–early September (Fig. 3), which coincided with a short growth period (12 and 13 days) immediately following a dosing (10 and 24 August). The isotopic signature of the tip segments of hair from the tail was similar to those of body hair at the end of the spring trials (Figs 1 and 3), likely reflecting incorporation of the last dose administered on 5 July. In contrast, the isotopic values of the two segments near the base were lower than those of body hair at the end of the autumn sampling, likely because the enriched glycine pool was diluted by the newly growing underfur of the body.

Discussion

Captive martens fed glycine enriched with ^{13}C, ^{15}N and ^{2}H exhibited substantive enrichment in their blood and hair compared with control animals. Although consumption of labeled amino acids resulted in elevation in isotopic values in body hair and blood during both seasons, we found that the rate of enrichment was higher in May–June for both tissues. In North America, martens begin moulting in late April and grow their winter coat during July–October (Markley & Bassett, 1942; Soutiere & Steventon, 1981; Rose *et al.*, 1984). It is likely that the high enrichment we observed during May–June, during a phase of predominantly hair loss, reflects the incorporation of the label via maintenance replacement of hair. During the peak of hair growth in autumn, when over half of the amino acids devoted to hair development are derived from recently consumed pools (Ayliffe *et al.*, 2004), the quality of the mark should have been the strongest. Potentially, the labeled amino acids were diluted by the many growing hairs in autumn and thus isotopic labeling during peak hair growth was not optimal. Our results indicate that for mammals with moult patterns similar to martens, the best period to offer isotopically enriched bait is summer (June–July). For other species, such as birds with different moult chronologies (Holmgren & Hedenström, 1995; Barta *et al.*, 2006), the incorporation of isotopically labeled amino acids into keratinous tissue may follow a different pattern.

Seasonal differences in slope coefficients for isotopic enrichment, particularly ^{2}H in whole blood, are difficult to explain. Because animals were acclimated to captivity for 5 weeks before feeding trials, diet was constant during the study (although we did not quantify the isotopic signature of each batch of commercial feed), and none of the martens exhibited substantial changes in body size (i.e. length or mass), it is unlikely that differential isotopic incorporation in whole blood was due to incomplete isotopic mixing, variation in diet quality or animal growth (Martínez del Rio & Wolf, 2005). Potentially, seasonal differences in incorporation rates of the isotopic label into blood were due to variation in isotopic values in either the drinking water or food (Hobson, Atwell & Wassenaar, 1999). Indeed, differences in incorporation rates were most apparent for ^{2}H, and control animals exhibited a change in blood values during autumn. However, isotopic values of ^{13}C and ^{15}N did not change as much in control animals, suggesting that seasonal differences were not the result of the isotopic composition of the diet alone. That the incorporation slopes for all isotopes were similar within seasons suggests another potential mechanism; the patterns we observed were caused by seasonal changes in tissue turnover rates. Seasonal changes in turnover rates have been reported for whole blood production in humans (Gunga *et al*., 2007) and muscle tissue of hibernating bears (Lohuis, Harlow & Beck, 2007). For example, Lohuis *et al*. (2007) observed that turnover rate of muscle in black bears *Ursus americanus* declined between autumn and winter, which was accompanied by a decline in the rate of change of ^{15}N. Whether seasonal changes in tissue turnover rates are common in non-hibernating vertebrates is unclear and merits further study.

Our results suggest that isotopically labeled glycine can be a cost-effective method to label small- to mid-sized vertebrates for studies of movement and dispersal. Indeed, a single dose of enriched bait for one marten currently costs approximately US$0.45 ($^{2}H$), $0.90 ($^{15}N$) and $6.60 ($^{13}C$), and the expenses of isotopic analyses with mass spectrometry are declining. It is likely that in the near future, additional amino acids will become available for administering isotopic labels. Notably, cysteine, a sulphur-containing amino acid and major component of keratinous tissues (Richards *et al*., 2003), is routed to hair growth (O'Connell & Hedges, 2001; Thomas *et al*., 2007) and, therefore, could provide another effective vehicle for isotopic enrichment. Currently, however, using this amino acid is cost-prohibitive. Still, the availability of multiple isotopes leads to the possibility of isotope combinations that can allow investigators to generate site-specific baits and isotopic marks. The number of unique permutations of isotopic marks will be $2^{n}-1$, where n is the number of isotopes of two forms used in the marking scheme. For example, if four elements were represented, each in two isotopic forms, 15 isotopic combinations, each including at least one form rare in nature, could be generated. Such an approach could provide an effective means for labeling multiple subpopulations and monitoring the movement and dispersal of individual animals across different habitats.

The safety of using enriched stable isotopes to study physiological processes in animals has been demonstrated (Koletzko, Sauerwald & Demmelmair, 1997). Unlike previously developed markers, which provide only a temporary mark (e.g. rhodamine B, sulfadimethoxine) or require the sacrifice of the animals for bone or tooth examination (e.g. tetracycline; Southey, Sleeman & Gormley, 2002), isotopic marks persist within the keratinous tissues (e.g. feathers, hair, scales) until they are moulted, and can be collected non-invasively. Non-invasive collection techniques for hair or feather (e.g. those shed or collected in hair traps and snares) have been developed and successfully employed (Mills, 2007) to sample wide-ranging, elusive and rare species, where live-capture can be impractical. Such collection techniques, primarily developed for DNA-based approaches, are cost-effective, and are well-suited for the method we describe. Isotopic labeling and analysis are further advantageous because isotopic composition of keratin, unlike DNA (Roon, Waits & Kendall, 2003), is resilient to environmental degradation (Macho *et al*., 1999). Finally, our cross-sectional analyses of hair reveal that the timing of bait consumption by the animal can be determined based on the temporal sequence of hair growth. Past studies have similarly shown that cross-sectional analyses of hairs can provide a chronology for changes in animal diet and dispersal (Cerling *et al*., 2006).

Our experiment demonstrated that isotopically enriched amino acids can be used to non-invasively and cost-effectively mark mid-sized vertebrates. We are now applying this method to a study with free-ranging animals. Researchers pursuing this technique for other vertebrate species will need to calculate the amount of dose required for desired enrichment levels and develop species-specific administration methods. Once such methods are established, isotopic labeling could facilitate marking large numbers of free-ranging vertebrates and tracking their movements at broad spatial scales, including the effects of fragmentation and metapopulation dynamics (McCullough, 1996).

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Mean length (± 1 SD) of guard hairs from three locations on captive martens, Laramie, Wyoming, 2006. Prior to 7 September, measures of hair growth from the tail reflected the hair from the previous molt, making measurements of new growth uncertain. Therefore, these data were omitted from our model of hair growth with the Gompertz function (Fig. 2). These data suggest that hair replacement on the body precedes that of the tail as previously reported (Bassett and Llewellyn 1949).

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